Truenat™

HBV

Chip-based Real Time PCR test for Hepatitis B Virus

1. INTENDED USE

Truenat™ HBV (REF 601090005/601090020) is a chip-based Real Time Polymerase Chain Reaction (qPCR) test for the quantitative estimation of the Hepatitis B Virus (HBV) in human blood / plasma specimen and aids in the diagnosis of infection with Hepatitis B Virus and in the estimation of viral load. Truenat™ HBV runs on the Truelab™ Uno Real Time micro PCR Analyzer.

2. INTRODUCTION

Hepatitis B infection is caused by the Hepatitis B Virus (HBV) that affects around two billion people globally, and causes around 6,00,000 deaths each year. The infection, which can be acute or chronic, occurs in the liver. Acute infections are mostly asymptomatic but sometimes may cause symptoms such as jaundice, extreme fatigue, vomiting and abdominal pain. A chronic infection can develop into cirrhosis of the liver and liver cancer. Transmission occurs from contact with infected blood or other body fluids through perinatal, transfusion, injection and sexual routes and through close contact with infected family members, especially in early childhood. Despite the availability of effective vaccination and antiviral drugs, Hepatitis B remains a major global health problem. The most common methods of diagnosing Hepatitis B are serology based assays and molecular tests. The HBV DNA is detectable about three weeks before the appearance of serological markers. HBV viral load quantitation by PCR is very useful for treatment initiation decisions and treatment monitoring. However PCR or Real Time PCR tests have so far been restricted to centralized reference laboratories as they require skilled manpower and elaborate infrastructure. Also the turnaround time for results could take a few days.

The Truelab™ Real Time micro PCR System enables decentralization and near patient diagnosis and treatment monitoring of Hepatitis B infection by making real time PCR technology rapid, simple, robust and user friendly and offering "sample to result" capability even at resource limited settings. This is achieved through a combination of lightweight, portable, mains / battery operated Truelab™ Uno Real Time micro PCR Analyzer and Trueprep™ MAG Sample Prep Device and room temperature stable Truenat™ chip-based Real time PCR test and Trueprep™ MAG Sample Prep kits so that even the peripheral laboratories with minimal infrastructure and minimally trained technician can easily perform these tests routinely in their facilities and report PCR results in less than an hour. Moreover, with these devices PCR testing can also be initiated in the field level, on site.

Truenat™ HBV is a disposable, room temperature stable, chip-based Real Time PCR test with dried down PCR reagents for performing Real Time PCR test for quantitative estimation of the Hepatitis B Virus and runs on the Truelab™ Uno Real Time micro PCR Analyzer. It requires only six (6) µL of purified DNA to be added to the reaction well for the analysis. The intelligent chip also carries test and batch related information including standard values for quantitation. The Truenat™ HBV chip also stores information of used test to prevent any accidental re-use of the test.

NOTE : Truelab™ / Truelab™ Uno / Trueprep™ MAG / Truepet™ / Truenat™ are all registered trademarks of Molbio Diagnostics (P) Limited.

The Truelab™ Real Time micro PCR Analyzer is protected by the following patents and patents pending: IN 2313/CHE/2007, WO 2009/047804 and corresponding claims of any foreign counterpart(s) thereof.

The Truenat™ micro PCR chip is protected by the following patents and patents pending: IN 2312/CHE/2007, WO 2009/047805 and corresponding claims of any foreign counterpart(s) thereof.

The Truenat™ HBV test is protected by the following patents and patents pending: IN 625/CHE/2011, WO 2012/117367 and corresponding claims of any foreign counterpart(s) thereof.

3. PRINCIPLE OF THE TEST

Truenat™ HBV works on the principle of quantitative Real Time Polymerase Chain Reaction. The DNA from the patient blood sample is first extracted using Trueprep™ MAG Sample Prep Device and Trueprep™ MAG Blood sample prep kit and six (6) µL of the purified DNA is then dispensed into the reaction well of the Truenat™ HBV chip-based Real Time PCR test. The Truenat™ HBV chip-based Real Time PCR test is then inserted in the Truelab™ Uno Real Time micro PCR Analyzer where thermal cycling takes place. A positive amplification causes the dual labeled fluorescent probe in the Truenat™ HBV chip-based Real Time PCR test to release the fluorophore in an exponential manner and the emitted light is then captured by the built-in opto-electronic sensor and displayed as amplification curve on the analyzer screen, on a real time basis during the test run. The Cycle threshold (Ct) is defined as the number of amplification cycles required for the fluorescent signal to cross the threshold (i.e. exceed the background signal). Ct levels are inversely proportional to the amount of target nucleic acid in the sample. (i.e. the lower the Ct level the greater is the amount of target nucleic acid in the sample). Ct value is linearly correlated with amount of target DNA present in the sample, enabling quantitative estimation of the HBV DNA. Standard curve values for every batch are preset in the Truenat™ HBV chip-based Real Time PCR test and the analyzer automatically compares these with the Ct value of the test sample to provide a quantitative result. In the case of negative samples, amplification does not occur and a horizontal amplification curve is displayed on the screen during the test run. At the end of the test run, a HBV "DETECTED" or "NOT DETECTED" result is displayed and in positive cases, Ct values and International Units (IU) per milliliter (IU/ml) is also displayed on the screen. Based on the Ct of the internal positive control (IPC), the validity of the test run is also displayed. The IPC is a full process control that undergoes all the processes the specimen undergoes - from extraction to amplification thereby validating the test run from sample to result. Absence of or shift of IPC Ct beyond a pre-set range in case of negative samples invalidates the test run. While IPC will co-amplify in most positive cases also, in some specimen having a high target load, the IPC may not amplify, however the test run is still considered valid. The results can be printed via Bluetooth using the Truelab™ micro PCR printer or transferred to the lab computer/or any remote computer via Wifi network or GPRS network. Upto 5000 results can be stored on the analyzer for future recall and reference.

4. TARGET SELECTION

The target sequence for this kit is part of the core/pre-core region of HBV genome. The region selected is specific to HBV and conserved across the HBV Genotypes.

5. CONTENTS OF THE Truenat™ HBV KIT

A. Individually sealed pouches, each containing a
   1. Truenat™ HBV chip-based Real Time PCR test for Hepatitis B Virus.
   2. DNase & RNase free pipette tip.
   3. Desiccant pouch.

B. Package Insert.

REF	601090005	601090020
Σ	5 T	20 T

6. STORAGE AND STABILITY

Truenat™ HBV chip-based Real Time PCR test is stable for one year from the date of manufacture if stored between 2-30 °C. It is also stable for upto three (3) months at temperatures up to 40° C. Avoid exposure to light.

7. MATERIALS REQUIRED BUT NOT PROVIDED WITH THE KIT

Truelab™ Real Time micro PCR Workstation (REF 603010001) consisting of

1. Trueprep™ MAG Sample Prep Device (REF 603040001).
2. Truelab™ Uno Real Time micro PCR Analyzer (REF 603020001).
3. Truelab™ micro PCR Printer (REF 603050001).
4. Truepet™ Precision Micropipettes - 6µL, 50µL, 100µL, 500µL, 1000µL. (REF 604010006 / 604020050 / 604030100 / 604040500 / 604051000).
5. Marker pen.

Also required additionally are: Trueprep™ MAG Blood Sample Prep kit (REF 602010050), Truenat™ Universal Control Kit (REF 601100008), DNase and RNase-free pipette tips (2 - 200µL / 200 - 1000µL microtips) with filter barrier, which may also be procured from Molbio (REF 604072200 / 604062010 respectively), Powder free disposable gloves, waste disposal container with lid.

8. SPECIMEN PREPARATION

Truenat™ HBV requires purified nucleic acids from blood/plasma specimen that are extracted using the Trueprep™ MAG Sample Prep Device and Trueprep™ MAG Blood Sample Prep Kit (Refer to the User Manual of Trueprep™ MAG Sample Prep Device and the package insert of Trueprep™ MAG Blood Sample Prep Kit for details).

9. SAFETY PRECAUTIONS

1. For *in vitro* diagnostic use only.
2. Bring all reagents and specimen to room temperature (20 - 30 °C) before use.
3. Do not use kit beyond expiry date.
4. Carefully read the User Manuals and package inserts of all the components of the Truelab™ Real Time micro PCR System before use.
5. All materials of human origin should be handled as though potentially infectious.
6. Do not pipette any material by mouth.
7. Do not eat, drink, smoke, apply cosmetics or handle contact lenses in the area where testing is done.
8. Use protective clothing and wear disposable gloves when handling samples and while performing sample extraction.

10. PROCEDURAL PRECAUTIONS

1. Check all packages before using the kit. Damage to the packaging does not prevent the contents of the kit from being used. However, if the outer packaging is damaged the user must confirm that individual components of the kit are intact before using them.
2. Do not perform the test in the presence of reactive vapours (e.g., from sodium hypochlorite, acids, alkalis or aldehydes) or dust.
3. While retrieving the Truenat™ HBV chip-based Real Time PCR test and the DNase & RNase free pipette tip from the pouch, ensure that neither bare hands nor gloves that have been used for previous tests run are used.

11. PROCEDURAL LIMITATIONS

1. Optimal performance of this test requires appropriate specimen collection, handling, storage and transport to the test site.
2. Though very rare, mutations within the highly conserved regions of the target genome where the Truenat™ assay primers and/or probe bind may result in the under-quantitation of or a failure to detect the presence of the concerned pathogen.
3. The instruments and assay procedures are designed to minimize the risk of contamination by PCR amplification products. However, it is essential to follow good laboratory practices and ensure careful adherence to the procedures specified in this package insert for avoiding nucleic acid contamination from previous amplifications, positive controls, or specimens.
4. A specimen for which the Truenat™ assay reports "Not Detected" cannot be concluded to be negative for the concerned pathogen. As with any diagnostic test, results from the Truenat™ assay should be interpreted in the context of other clinical and laboratory findings.

12. CLEANING AND DECONTAMINATION

1. Spills of potentially infectious material should be cleaned up immediately with absorbent paper tissue and the contaminated area should be decontaminated with disinfectants such as 0.5% freshly prepared sodium hypochlorite (10 times dilution of 5% sodium hypochlorite (household bleach) before continuing work.
2. Sodium hypochlorite should not be used on an acid-containing spill unless the spill-area is wiped dry first. Materials used to clean spills, including gloves, should be disposed off as potentially bio-hazardous waste e.g. in a biohazard waste container.

13. TEST PROCEDURE

(Please also refer Section 4 in the Truelab™ Uno Real Time micro PCR Analyzer user manual)

1. Switch on the Truelab™ Uno Real Time micro PCR Analyzer touch screen.
2. Select user and enter password.
3. Select the test profile for "HBV" on the Analyzer screen.
4. Enter the patient details as prompted in the Truelab™ Uno Real Time micro PCR Analyzer screen.
5. Press Start Reaction.
6. Press the eject button to open the chip tray.
7. Open a pouch of Truenat™ HBV and retrieve the chip-based Real Time PCR test.
8. Label the chip with the patient ID using a marker pen at the space provided on the back side of the chip.
9. Place the Truenat™ HBV chip-based Real Time PCR test on the chip tray without touching the white reaction well. The reaction well should be facing up and away from the Analyzer. Gently press the chip to ensure that it is seated in the chip tray properly.
10. Using the filter barrier tip provided in the pouch, pipette six (6) µL of the purified DNA from the Elute Collection Tube into the centre of the white reaction well. Take care not to scratch the

internal well surface and not to spill elute on the outside of the well.

11. Slide the chip tray containing the Truenat™ HBV chip-based Real Time PCR test loaded with the sample, into the Truelab™ Uno Real Time micro PCR Analyzer.
12. Press the power button on the Analyzer to turn it on. The green LED should glow.
13. Press Done on the "Please Load Sample" Alert message.
14. At the end of the test, observe the optical plot for any irregularities (Refer to the Truelab™ Uno Real Time micro PCR Analyzer manual).
15. Read the result from the screen.
16. Press the power button on the Analyzer to turn it off. The green LED should stop glowing.
17. Take out the Truenat™ HBV chip-based Real Time PCR test at end of the test and dispose it off as per the section on "Disposal and Destruction" (Section 16).
18. Turn on Truelab™ micro PCR printer and select print on the screen for printing out hard copy of the results. Test results are automatically stored and can be retrieved any time later. (Refer to the Truelab™ Uno Real Time micro PCR Analyzer manual).
19. Switch off the Truelab™ Uno Real Time micro PCR Analyzer touch screen.

14. RESULTS & INTERPRETATIONS

Two amplification curves are displayed on the Truelab™ Uno Real Time micro PCR Analyzer screen to indicate the progress of the test. Both the target and the internal positive control (IPC) curves will take a steep, exponential path when the fluorescence crosses the threshold value in case of positive samples. The Ct will depend on the number of viral genomes in the sample. The target curve will remain horizontal throughout the test duration and the IPC curve will take an exponential path in case of negative samples. In case the IPC curve remains horizontal in a negative sample, the test is considered as Invalid. At the end of the test run, the results screen will display "DETECTED" for Positive result or "NOT DETECTED" for Negative result. The result screen would also display the Ct value and the IU/ml for positive specimen. The result screen also displays the validity of the test run as "VALID" or "INVALID". Invalid samples have to be repeated with fresh specimen from the sample preparation stage. While IPC will co-amplify in most positive cases also, in some specimen having a high target load, the IPC may not amplify, however the test run is still considered valid.

15. QUALITY CONTROL PROCEDURES

To ensure that the Truelab™ Uno Real Time micro PCR Analyzer is working accurately, run positive and negative controls from time to time. The Truenat™ Universal Control Kit containing Positive Control and Negative Control must be ordered separately. It is advisable to run controls under the following circumstances: ? Whenever a new shipment of test kits is received. ? When opening a new test kit lot. ? If the temperature of the storage area falls outside of 2-30° C. ? By each new user prior to performing testing on clinical specimen.

16. DISPOSAL AND DESTRUCTION

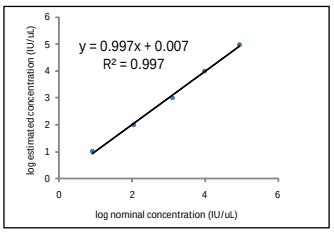
1. Submerge the used Truenat™ HBV chip-based Real Time PCR test in freshly prepared 1% sodium hypochlorite solution for 20 minutes before disposal as per the standard medical waste disposal guidelines.
2. Disinfect the solutions and/or solid waste containing biological samples before discarding them according to local regulations.
3. Samples and reagents of human and animal origin, as well as contaminated materials, disposables, neutralized acids and other waste materials must be discarded according to local regulations after decontamination by immersion in a freshly prepared 0.5% of sodium hypochlorite for 30 minutes (1 volume of 5% sodium hypochlorite for 10 volumes of contaminated fluid or water).
4. Do not autoclave materials or solutions containing sodium hypochlorite.
5. Chemicals should be handled in accordance with Good Laboratory Practice and disposed off according to the local regulations.

17. SPECIFIC PERFORMANCE CHARACTERISTICS

ASSAY RANGE AND LIMIT OF DETECTION

The assay is linear over 6 log DNA dilutions and has a lower limit of detection of 100 IU/ml (Based on run data from 3rd WHO International Standard for Hepatitis B Virus for Nucleic Acid Amplification Techniques, NIBSC code: 10/264).

To ascertain the ability of the Truenat™ HBV test to provide accurate viral loads, DNA standards containing known amount of virus (loads provided as IU/ul) were run on the Truelab™ analyzer. The graph above displays the viral loads estimated by the Truenat™ HBV test plotted against the expected viral loads. The Truenat™ HBV viral load values match closely (R^2= 0.99) with the expected values.



ANALYTICAL SENSITIVITY AND SPECIFICITY

To determine the sensitivity and specificity of the Truenat™ HBV probe and primers, a characterized panel containing 50 positive and 50 negative samples was used. Samples were first tested using a commercially available PCR kit for detection of Hepatitis B virus. The same DNA was tested using the Truenat™ HBV chip-based Real Time PCR Test. Both methods gave concordant results with all 100 samples being correctly identified as positive or negative.

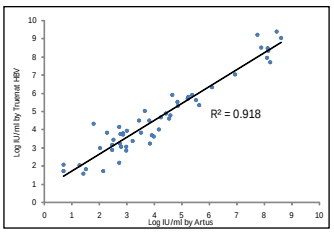
ANALYTICAL EXCLUSIVITY

To establish the absence of cross-reactivity of the Truenat™ HBV probe and primer set with other common pathogens, nucleic acids were extracted from cultures of the following organisms:
?Hepatitis C virus ? Dengue Virus Serotype 1, 2, 3 and 4 ? Chikungunya Virus ? *Plasmodium falciparum* ? *Plasmodium vivax* ? Salmonella spp. ? *Klebsiella pneumoniae* ? H1N1 virus ? *Escherichia coli* ? Human Immunodeficiency virus. The extracted nucleic acid samples were tested using Truenat™ HBV chips and all samples tested negative indicating that the Truenat™ HBV test is specific for Hepatitis B virus.

CLINICAL VALIDATION

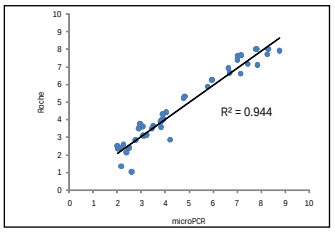
Study 1:

The Truenat™ HBV was compared with a CE-marked kit for detection of Hepatitis B virus. The study involved 87 samples and it was found that the results from both methods correlated well. For the positive samples, a Bland-Altman analysis found no significant difference between viral loads obtained by the two methods.



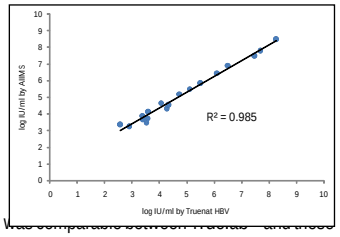
Study 2:

The Truenat™ HBV test was compared with an FDA approved test for detection of Hepatitis B virus. The study involved a characterized sample panel containing 50 samples. The Truenat™ HBV was able to detect all 41 positive samples and the viral loads obtained were not significantly different from the viral loads determined by the FDA approved test.



Study 3:

The performance of the Truenat™ HBV was compared against an in-house assay of a tertiary care hospital in India. The hospital's real-time PCR assay for Hepatitis B was calibrated using the 3rd W.H.O. International Standard for Hepatitis B Virus for Nucleic Acid Amplification Techniques. The study involved 25 samples of which 18 were detected as positive by the in-house PCR assay. The Truenat™ HBV was able to detect all positive samples and viral loads obtained matched closely with those determined by the hospital's assay.



Thus the test performance and viral load quantification was comparable between Truelab and those systems.

REPRODUCIBILITY AND PRECISION

Assay reproducibility and precision was determined across different users, analyzers and reagent lots. Serial log dilutions of HBV DNA were used for this purpose. The same sample panel was provided to 3 users, who ran it on 3 different Truelab™ analyzers with 3 different lots of Truenat™ HBV chip-based Real Time PCR tests. High inter-user, inter-analyzer and inter-lot reproducibility was observed (avg. standard deviation = 0.25 Ct). No significant difference was observed in the Ct values obtained from different users, analyzers or lots.

INTERFERING SUBSTANCES

Effect of interferences of elevated blood parameters such as lipid, cholesterol and triglycerides were evaluated in this study. Archived negative blood samples with higher level of serum parameters were spiked with known amounts of a positive clinical specimen. These samples were then subjected to nucleic acid extraction and the eluted DNA was run on the Truelab™ analyzer. To determine the effect, same quantity of positive clinical specimen was also spiked into normal blood, and processed as above.

Duplicates runs were performed for each samples and the table shows the average Cts. No significant difference was observed in the log IU/ml values (Avg. = 5.93, Std. Dev. = 0.36) indicating that the presence of elevated blood parameters does not interfere significantly with the detection of Hepatitis B virus by Truenat™ HBV.

CARRY OVER EFFECT

To determine whether the Truenat™ HBV chip-based Real Time PCR test showed any signs of carryover of PCR products between runs, alternating runs of positive high titre samples and negative samples were performed in duplicates. 10 positive and 10 negative samples were used for this study. All positive samples were accurately detected and all negative samples were undetected indicating the absence of carryover of PCR products between runs.

18. REFERENCES

1. http://www.who.int/mediacentre/factsheets/fs204/en/
2. http://www.cdc.gov/hepatitis/hbv/pdfs/hepbgeneralfactsheet.pdf.
3. Fryer JF, Heath AB, Wilkinson DE, Minor PD and the collaborative study group. Collaborative study to evaluate the proposed 3rd WHO International Standard for hepatitis B virus (HBV) for nucleic acid amplification technology (NAT)-based assays. WHO ECBS Report 2011; WHO/BS/2011.2170
4. Abe, Aki, et al. "Quantitation of hepatitis B virus genomic DNA by real-time detection PCR." *Journal of Clinical Microbiology* 37.9 (1999): 2899-2903.
5. Chen, Ren Wei, et al. "Real-time PCR for detection and quantitation of hepatitis B virus DNA." *Journal of medical virology* 65.2 (2001): 250-256.
6. Brechtbuehl, K., et al. "A rapid real-time quantitative polymerase chain reaction for Hepatitis B virus." *Journal of virological methods* 93.1 (2001): 105-113.

SYMBOL KEYS:

Refer package insert for instructions before use	IVD	*In vitro* Diagnostic Test. Not for medicinal use.	2°C 30°C	Store between 2-30°C	REF	Catalogue Number	For single use only
Manufacturer		Date of Manufacture		Date of Expiry	LOT	Batch Number / Lot Number	Σ	Contains sufficient for <n> tests

Manufactured by:

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